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Scanning Electron Microscopy of the Chick Embryo

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Internal anatomical features of developing chick embryos can be examined using the scanning electron microscope in embryos that have been sectioned at varying levels. Standard paraffin sectioning techniques are used. After removal of paraffin, embryos are dried by the critical point method and examined. The resulting scanning electron micrographs have proved to be exceptionally useful in teaching anatomy of embryos.

INTRODUCTION

Scanning electron microscopy has found abundant application in studies of the fine structure of the surfaces of biological specimens. The introduction of the critical point drying technique has allowed observations to be performed on cells, tissues, and other soft specimens (Boyde and Wood, 1969; Grey, 1972; Nowell *et al.*, 1972; Porter, 1972; Rubin, *et al.*, 1972; Waterman, 1972) with a minimum of distortion during drying. Usually, the subject is fixed in aldehyde, dehydrated, and dried from liquid CO_2 at its critical point.

We have applied these techniques in the production of scanning electron micrographs which are used in teaching microanatomy of embryos in a beginning course in embryology. In order to visualize internal features, embryos were sectioned to varying depths and the cut surface was examined. Clean even cuts were produced by sectioning paraffin-embedded embryos on a microtome. The paraffin was then removed and the embryo was subjected to critical point drying. An advantage of this procedure is that the plane of section can be determined very precisely.

MATERIALS AND METHODS

The method used was essentially the [same] as that described in Armstrong [...]. Carefully staged embryos were removed from the yolk mass using the filter-paper ring technique and were fixed in 2% glutaraldehyde in phosphate buffer. Fixed embryos were dehydrated in ethanol, cleared in toluene, and embedded in paraffin. During dehydration, embryos were stained with eosin. For sectioning, embryos were oriented carefully on the microtome and were sectioned to the desired depth. During sectioning, each succeeding section was examined under a dissecting microscope until the desired depth of sectioning was achieved. The paraffin was removed from the remainder of the embryo in several changes of warm toluene and the embryo was then transferred through absolute ethanol to amyl acetate. Critical point drying was by the technique of Anderson (1951). Dried embryos were mounted on stubs and coated with gold in a vacuum evaporator. In order to ensure that the embryos were displayed face-on in the scanning electron microscope for photography (i.e., to avoid distortion due to parallax), drawings were made of the cut faces of carefully oriented embryos using a Wild M-5 stereomicroscope with drawing attachment. The orientation of the embryo in the scanning electron microscope could then be adjusted until its dimensions corresponded to those of the drawing. A Cambridge Stereoscan scanning electron microscope operated at 10 kV was used throughout the course of this study.

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RESULTS AND DISCUSSION

Using the techniques described above, we have produced a series of scanning electron micrographs illustrating the anatomical features of chick embryos at varying levels in the embryo and in embryos of varying stages of development. The series has been an invaluable aid in teaching embryonic anatomy since it provides a view unobtainable by any other means. In order to understand anatomy, it is necessary to have a three-dimensional picture of anatomical relationships. Difficult as this may be when studying the anatomy of large specimens, the problem is considerably more difficult when one studies something as small as a young chick embryo. In this case, internal anatomy is traditionally studied with a microscope from serial two-dimensional sections. Many beginning students find that their greatest difficulty comes in constructing a picture of the embryo in three dimensions from these series of two-dimensional sections.

We have found our series of scanning electron micrographs to be most useful in overcoming this problem since the scanning electron microscope enables one to produce pictures with a considerable depth of focus to allow the direct visualization of tissues and organs in three dimensions. Useful adjuncts to the procedure include the dissection of embryos (followed by the application of a second coat of gold) and their photography from different angles to reveal further the relations of structures to one another (Fig. 4 and 5, 6 and 7) and the production of stereo-pairs of photographs. In sum, the clarity, high resolution, and ample depth of focus of the scanning electron microscope permits the demonstration of anatomical features in a fashion that makes

FIG. 1. Right half of a 33-hr chick embryo. Visible are the major divisions of the brain (prosencephalon (*pros*) with right optic vesicle, mesencephalon (*mes*), and rhombencephalon (*rhom*)), notochord (*n*), foregut (*f*), anterior intestinal portal (*aip*), left vitelline vein (*vv*), heart (*h*), and ventral aorta (*va*). × 100.

FIG. 2. Right half of the head and pharynx region of a 72-hr chick embryo. The prosencephalon is subdivided into the telencephalon (*tel*) and diencephalon (*dien*), from which arises the stalk of the optic vesicle (*o*). Most of the mesencephalon and the metencephalon are out of view at the top of the picture. The myelencephalon can be distinguished by the presence of neuromeres (*neu*) in its ventral wall. In this picture, the digestive system is represented by the stomodeum (*s*) with Rathke's pouch (*R*) as a dorsal diverticulum and the pharynx (*p*) with pharyngeal pouches 1–4. The separation between pharynx and stomodeum is demarcated by remnants of the pharyngeal plate (*pp*). The circulatory system is represented by the ductus venosus (*dv*), which has been invaded by cords of liver tissue, the sinus venosus (*sv*), the atrium (*at*), the ventricle (*v*), the conus (*c*), the base of aortic arches 3 and 4 (*aa*), and the dorsal aorta (*da*). An idea of the growth of the embryo between 33 and 72 hr can be had by comparing Figs. 1 and 2. Both are printed at the same magnification. × 100.

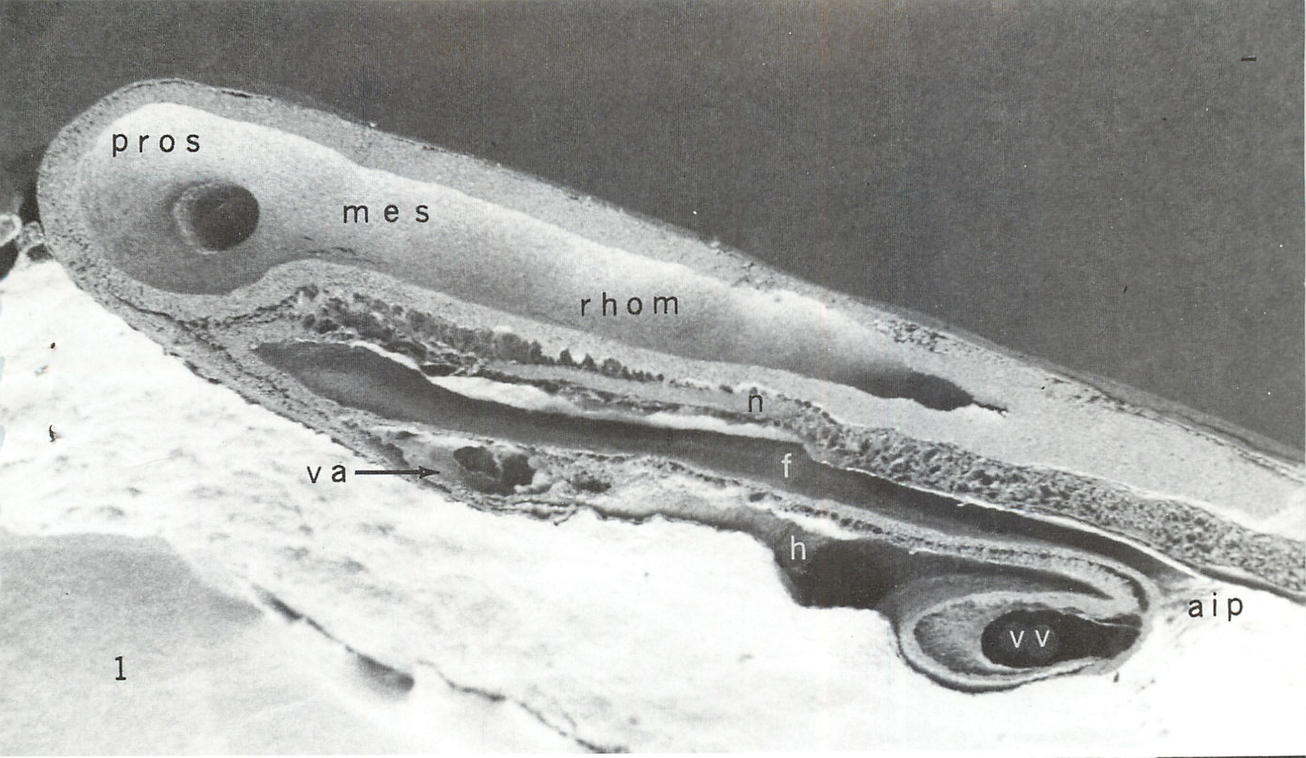
FIG. 3. Frontal view of the floor of pharynx of a 72-hr embryo. The plane of section cuts the diencephalon (*dien*), eyes (*o* = eyecup, *le* = lens), aortic arches 1 (*1*) and 2 (*2*), the paired dorsal aortae (*da*), anterior cardinal veins (*ac*), spinal cord (*sc*), and notochord (*n*). The depression in the floor of the pharynx is the thyroid primordium, and the columns of tissue on each side of the pharynx are the pharyngeal arches 2 and 3. The yolk sac (*y*), chorion (*ch*), and amnion (*am*) surround the embryo. × 121.

FIG. 4. In this specimen, the plane of section lies deeper than in Fig. 3. On the left side of the embryo is the exterior surface of the atrium (*at*) and on the right side, just grazed by the section, is the conus (*c*). Between the two, the base of the aortic arches (*aa*) can be seen. The digestive system is represented by the esophagus (*e*) and laryngotracheal groove (*ltg*). This embryo is unusual in that it lies with its right side in contact in the yolk sac. × 80.

FIG. 5. The same embryo as Fig. 4. The head was dissected away, the specimen was recoated with gold, and the embryo was rotated and tilted up to allow a view of the heart looking from the front of the embryo. *at*, atrium; *v*, ventricle; *c*, conus. × 70.

FIG. 6. View of the anterior intestinal portal of a 72-hr chick embryo. *pc*, posterior cardinal veins, *W*, Wolffian duct, *wb*, wing bud, *coel*, coeloem, *som*, somite. Other designations the same as in preceeding figures. × 70.

FIG. 7. View of the right side of an embryo sectioned from back to front to the level of the anterior intestinal portal. The extraembryonic membranes have been removed. Shown are the nasal placodes (*np*), the bulge of the eye, pharyngeal grooves (*pg*), and the ventricle of the heart (*v*). × 52.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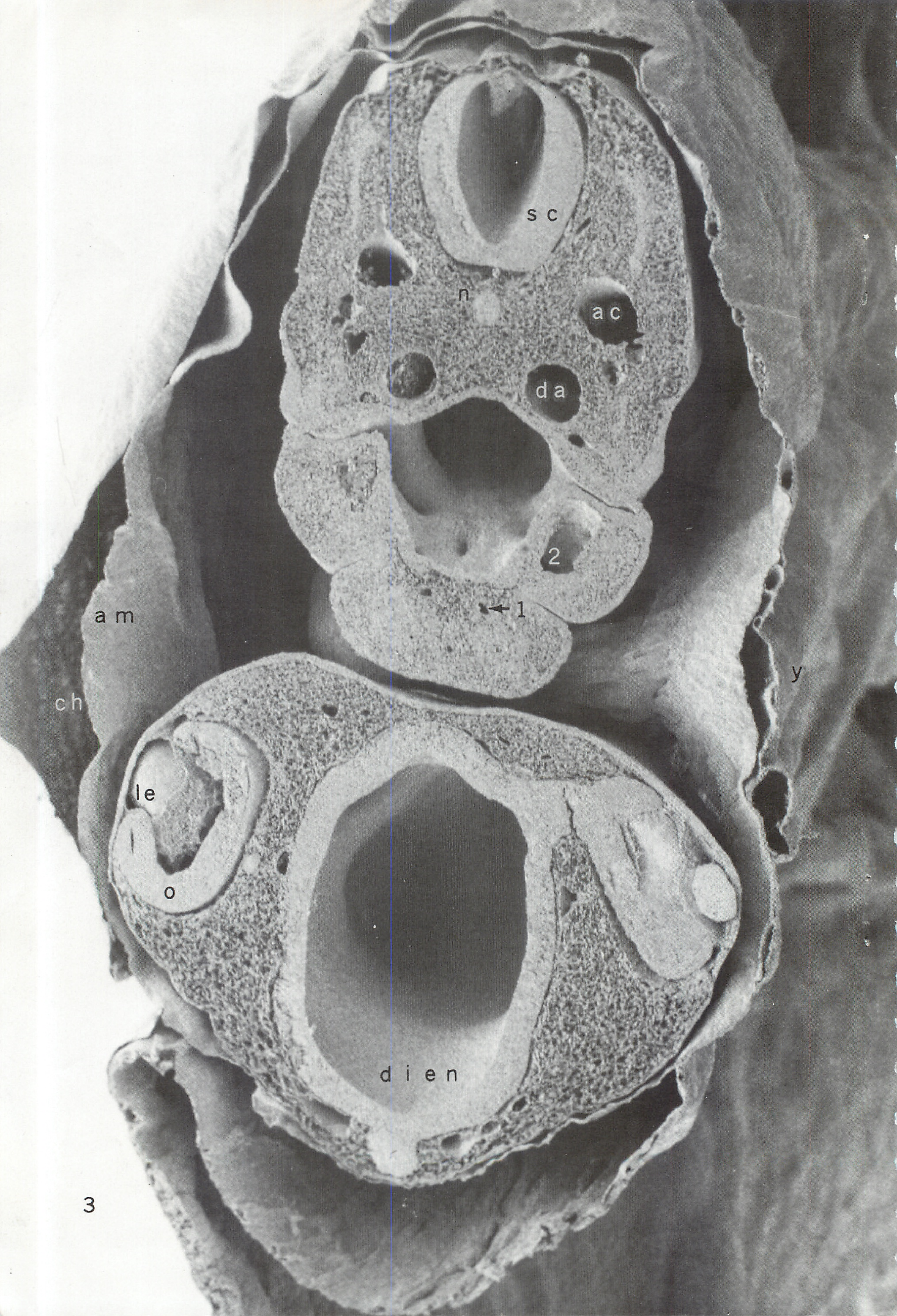

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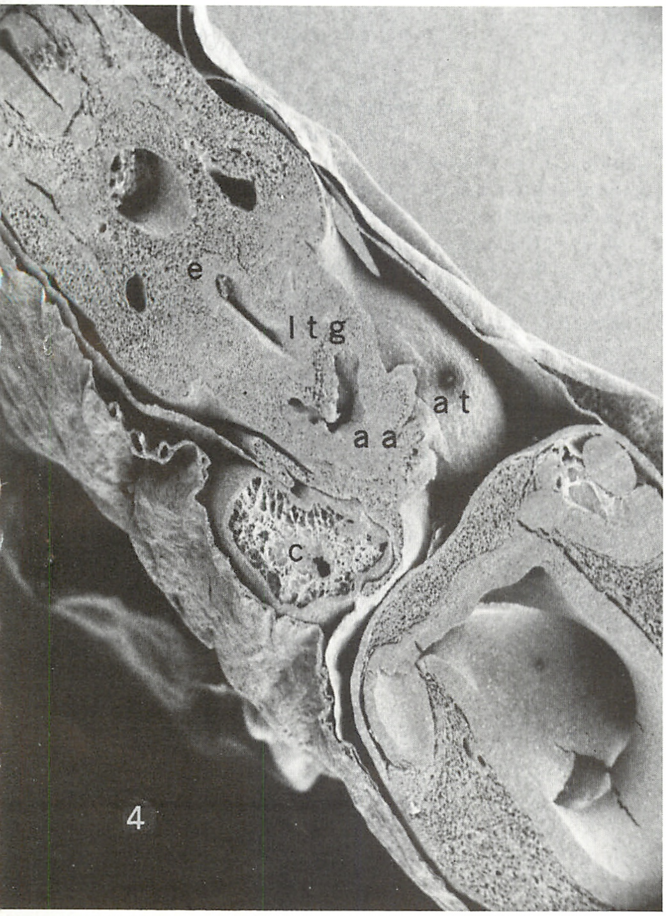
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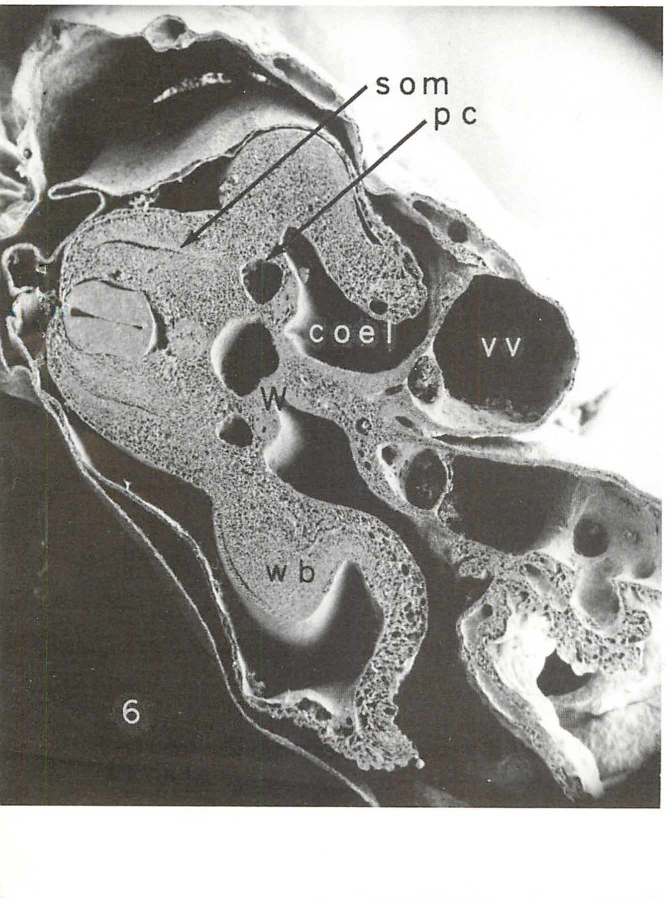
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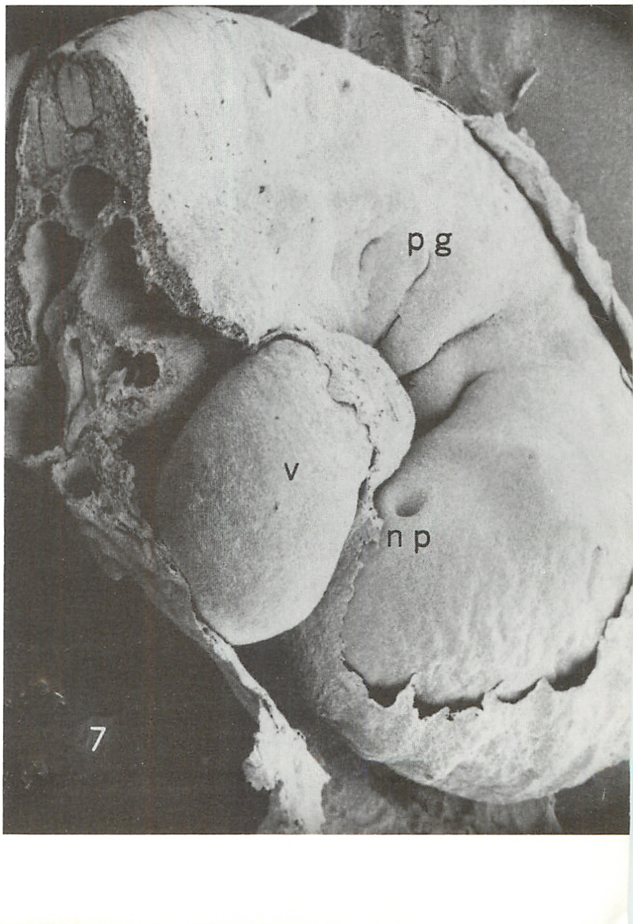
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the teaching of embryonic anatomy both easier and more successful.

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REFERENCES

ANDERSON, T. F. (1951). Techniques for the preservation of three-dimensional structure in preparing specimens for the electron microscope. *Trans. N. Y. Acad. Sci.* **13,** 130–134.

ARMSTRONG, P. B. (1971). A scanning electron microscope technique for study of the internal microanatomy of embryos. *Microscope* **19,** 281–284.

BOYDE, A., and WOOD, C. (1969). Preparation of animal tissues for surface-scanning electron microscopy. *J. Microsc.* **90,** 221–249.

GREY, R. D. (1972). Morphogenesis of intestinal villi. I. Scanning electron microscopy of the duodenal epithelium of the developing chick embryo. *J. Morphol.* **137,** 193–214.

NOWELL, J. A., PANGBORN, J., and TYLER, W. S. (1972). Stabilization and replication of soft tubular and alveolar systems. A scanning electron microscope study of the lung. *In*: Scanning Electron Microscopy (1972). Proceedings of the Workshop on Biological Specimen Preparation Techniques for Scanning Electron Microscopy—IIT Research Inst., part II, pp. 305–312.

PORTER, K. R. (1972). The surfaces of cells and miscellaneous soft tissues as revealed by scanning electron microscopy. *Anat. Rec.* **172,** 441–442.

RUBIN, R. W., EVERHART, L. P., PORTER, K. R., PRESCOTT, D. M., and FRYE, J. (1972). Changes in form and surface morphology of CHO cells in response to progression through the cell cycle and intercellular contact. *J. Cell Biol.* **55,** 222a.

WATERMAN, R. E. (1972). Use of the scanning electron microscope for observation of vertebrate embryos. *Develop. Biol.* **27,** 276–281.